OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

Mag-Bind® PCR Clean Up 96 Kit

M1382-00	1 x 96
M1382-01	4 x 96
M1382-03	100 x 96

June 2013

For research use only.Not intended for diagnostic testing.

Mag-Bind® PCR Clean Up 96 Kit

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Manual Revision: June 2013

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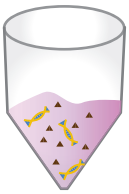
Introduction and Principle

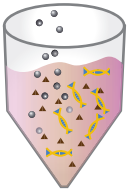
Omega Bio-tek's Mag-Bind® PCR Clean Up 96 Kit allows rapid and reliable isolation of PCR* products with high recovery rates. The system combines Omega Bio-tek's proprietary chemistries with the reversible nucleic acid-binding properties of magnetic beads that selectively bind PCR amplicons 100 bp and larger and eliminate excess nucleotides, primers, and small, non-targeted amplification products, such as primer dimers. This kit is designed for both manual and fully automated purification of PCR samples. Purified PCR fragments can be used for microarrays, automated fluorescent DNA sequencing, restriction enzyme digestion, and other applications.

The Mag-Bind® PCR Clean Up 96 Kit's magnetic particles technology provides a better solution for nucleic acid purification compared to centrifugation and vacuum-based technologies. The product can be easily scaled up while providing very user-friendly handling procedures. If using Mag-Bind® PCR Clean Up for the first time, please read this booklet to become familiar with the procedures. PCR products are first mixed with Mag-Bind® PCR Clean Up Mastermix which selectively binds PCR products to the Mag-Bind® Particles CNR. With two rapid wash steps, trace contaminants such as nucleotides, primers and small, non-targeted amplification products are removed. Pure DNA is eluted in Elution Buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

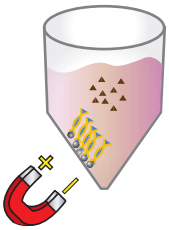
Illustrated Protocol



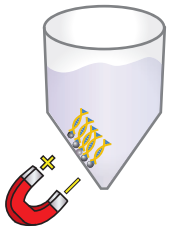
Measure the PCR Reaction



Add Mag-Bind® PCR Clean Up Mastermix

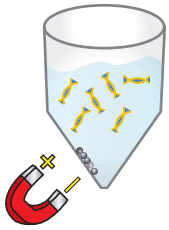


Magnetize and Remove Supernatant



Wash Twice with 70% Ethanol

Dry



Elute DNA

Kit Contents

Product Number	M1382-00	M1382-01	M1382-03
Preps	1 x 96*	4 x 96*	100 x 96*
CB Buffer	8 mL	40 mL	1000 mL
Mag-Bind® Particles CNR	300 µL	2.2 mL	50.8 mL
Elution Buffer^	10 mL	50 mL	1000 mL
User Manual	✓	✓	✓

* Based upon 25 µL PCR reactions
^ 10 mM Tris HCl pH 8.5

Storage and Stability

All of the Mag-Bind® PCR Clean Up 96 Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Mag-Bind® Particles CNR should be stored at 2-8°C for long-term use. All remaining components should be stored at room temperature.

Mag-Bind® PCR Clean Up 96 Kit- 96-well Plate Protocol

Materials and Equipment to be Supplied by User:

- 96-well PCR plate containing PCR samples (up to 100 µL/well)
- Magnetic Separation Device (Recommended Alp Aqua A001322)
- Multichannel pipettor
- Reservoirs
- Sealing film
- 96-well microplate/PCR for elution
- 70% ethanol
- Optional: Oven capable of 37°C

1. Read the manufacturer's instruction manual for the magnetic separation device, if provided.

2. Place the 96-well PCR plate on the bench and measure the volume of the PCR reaction. Determine if transferring the sample to a processing plate is required. If necessary, transfer the PCR reactions to a 96-well microplate.

   Note: PCR Reactions >50 µL will need to be transferred to a processing plate. If processing in a PCR plate, a magnet compatible with PCR plates must be used. (Recommended Alp Aqua A001322)

3. Prepare a Mag-Bind® PCR Mastermix containing Mag-Bind® Particles CNR and CB Buffer according to the table below. Vortex the Mag-Bind® Particles CNR for 1 minute immediately prior to preparing the mastermix.

PCR Reaction Volume (µL)	Preps	Mag-Bind® Particles CNR (µL)	CB Buffer (µL)	Total Volume (µL)
25	96	264	5280	5544
10	96	106	2112	2218

4. Shake the Mag-Bind® PCR Clean Up Mastermix to resuspend any Mag-Bind® Particles CNR that may have settled.

5. Add 2 volumes Mag-Bind® PCR Clean Up Mastermix to each well following the table below.

PCR Reaction Volume (µL)	Mag-Bind® PCR Clean Up Mastermix (µL)
10	20
25	50
50	100
100	200

6. Pipet up and down 5-10 times.

7. Let sit at room temperature for 5 minutes.

8. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

9. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CNR.

10. Add 200 µL 70% ethanol to each well.

11. Let sit at room temperature for 1 minute. It is not necessary to resuspend the Mag-Bind® Particles CNR.

12. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CNR.

13. Repeat Steps 10-12 for a second 70% ethanol wash step.

14. Leave the plate on the magnetic separation device for 5 minutes to air dry the Mag-Bind® Particles CNR. Remove any residue liquid with a pipettor.

    Note: It is important to dry the Mag-Bind®Particles CNR before elution. Residual ethanol may interfere with downstream applications.

    Optional: Incubate the plate at 37°C. Incubation can speed up evaporation.

15. Remove the plate from magnetic separation device.

16. Add 30-40 µL Elution Buffer to each well.

17. Pipet up and down 20 times or vortex for 30 seconds.

18. Let sit at room temperature for 2-3 minutes.

19. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

20. Transfer the cleared supernatant containing purified DNA to a new 96-well microplate and seal with non-permeable sealing film.

21. Store the plate at 2-8°C if storage is only for a few days. For long-term storage, samples should be stored at -20°C.

Mag-Bind® PCR Clean Up 96 Kit - 384-well Plate Protocol

Materials and Equipment to be Supplied by User:

- 384-well PCR plate containing PCR samples (up to 5 µL/well)
- Magnetic separation device for 384-well PCR plates (Recommended Alp Aqua A001222)
- Multichannel pipettor
- Reservoirs
- Sealing film
- 70% ethanol
- 384-well plate for elution
- Optional: Oven capable of 37°C

1. Read the manufacturer's instruction manual for the magnetic separation device, if provided.

2. Place the 384-well PCR plate on the bench and measure the volume of the PCR reaction. Transfer the sample to a skirted 384-well PCR plate. If a 10 µL reaction is used, then samples will need to be transferred to a 384-well deep-well plate for processing.

3. Prepare a Mag-Bind® PCR Mastermix containing Mag-Bind® Particles CNR and CB Buffer according to the table below. Vortex the Mag-Bind® Particles CNR for 1 minute immediately prior to preparing the mastermix.

PCR Reaction Volume (µL)	Preps	Mag-Bind® Particles CNR (µL)	CB Buffer (µL)	Total Volume (µL)
5	384	213	4250	4463

4. Shake the Mag-Bind® PCR Clean Up Mastermix to resuspend any Mag-Bind® Particles CNR that may have settled.

5. Add 2 volumes Mag-Bind® PCR Clean Up Mastermix to each well following the table below.

PCR Reaction Volume (µL)	Mag-Bind® PCR Clean Up Mastermix (µL)
5	10

6. Pipet up and down 5-10 times.

7. Let sit at room temperature for 5 minutes.

8. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

9. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CNR.

10. Add 25 µL 70% ethanol to each well.

11. Let sit at room temperature for 1 minute. It is not necessary to resuspend the Mag-Bind® Particles CNR.

12. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CNR.

13. Repeat Steps 10-12 for a second 70% ethanol wash step.

14. Leave the plate on the magnetic separation device for 5 minutes to air dry the Mag-Bind® Particles CNR. Remove any residue liquid with a pipettor.

    Note: It is important to dry the Mag-Bind® Particles CNR before elution. Residual ethanol may interfere with downstream applications.

    Optional: Incubating the plate at 37°C can speed up the evaporation.

15. Remove the plate from magnetic separation device.

16. Add 20 µL Elution Buffer to each well.

17. Pipet up and down 20 times or vortex for 30 seconds.

18. Let sit at room temperature for 2-3 minutes.

19. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR is completely cleared from solution.

20. Transfer the cleared supernatant containing purified DNA to a new 384-well microplate and seal with non-permeable sealing film.

21. Store the plate at 2-8°C if storage is only for a few days. For long-term storage, samples should be stored at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **800-832-8896**.

Possible Problems and Suggestions

Problem	Cause	Solution
Low yield	Low PCR product yield	Increase the number amplification cycles for PCR.
	Smaller PCR product size	Small PCR fragments normally give lower yield.
	Ethanol residue	During the drying step, remove any liquid from bottom of the well.
	Particle loss during the procedure	Increase magnetization time. Aspirate slowly.
	DNA remains bound to beads	Increase elution volume.
	Incomplete resuspension of the beads during elution	Vortex or pipet up and down to fully resuspend the beads.
Problem	**Cause**	**Solution**
Primer carryover	Insufficient wash of the particles	Wash the beads one more time with 70% ethanol.
Problem	**Cause**	**Solution**
Non-specific amplification products were not removed	The size of the non-specific amplification products are larger than 100 bp	Non-specific amplification products larger than 100 bp are not efficiently removed from PCR products.
Problem	**Cause**	**Solution**
Problems in downstream applications	Salt carryover	70% ethanol must be stored at room temperature.
	Ethanol carryover	Ensure the beads are completely dried before elution.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
Magnetic Separation Device for 96-well Plates	A001322
Magnetic Separation Device for 384 -well Plates	A001222
Elution Buffer (100 mL)	PDR048
96-well Microplate (500 µL) (25/pk)	EZ9604-02
Multichannel Disposable Reservoirs (100/pk)	AC1331-01
Sealing Film (100/box)	AC1200-01